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*Published in:*  
Brain-research

*DOI:*  
[10.1016/S0006-8993\(03\)03320-1](https://doi.org/10.1016/S0006-8993(03)03320-1)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2003

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

de Wilde, MC., Höggyes, E., Kiliaan, AJ., Farkas, T., Luiten, PGM., Farkas, E., Wilde, M. C. D., Höggyes, E., & Kiliaan, A. J. (2003). Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats. *Brain-research*, 988(1-2), 9 - 19. [https://doi.org/10.1016/S0006-8993\(03\)03320-1](https://doi.org/10.1016/S0006-8993(03)03320-1)

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## Research report

## Dietary fatty acids alter blood pressure, behavior and brain membrane composition of hypertensive rats

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Accepted 10 July 2003

## Abstract

The beneficial effect of dietary n-3 polyunsaturated fatty acids (PUFAs) on developing hypertension has been repeatedly demonstrated. However, related changes in brain membrane composition and its cognitive correlates have remained unclear. Our study aimed at a comprehensive analysis of behavior and cerebral fatty acid concentration in hypertension after long-term PUFA-rich dietary treatment. Hypertensive and normotensive rats were provided a placebo, or one of two PUFA-enriched diets with a reduced (n-6)/(n-3) ratio for 75 weeks. Exploratory behavior and spatial learning capacity were tested. Systolic blood pressure (BP) was repeatedly measured. Finally, brain fatty acid composition was analyzed by gas chromatography. Hypertensive rats exhibited more active exploration but impaired spatial learning compared to normotensives. Both diets reduced BP, increased PUFA and monounsaturated fatty acid (MUFA) concentration, and reduced saturated fatty acid content in brain. The level of cerebral PUFAs and MUFAs was lower in hypertensive than in normotensive rats. Furthermore, BP positively, while spatial learning negatively correlated with cerebral (n-6)/(n-3) PUFA ratio. We concluded that regular n-3 PUFA consumption could prevent the development of hypertension, but reached only a very delicate improvement in spatial learning. Furthermore, we consider a potential role of metabolically generated MUFAs in the beneficial effects of PUFA supplementation.

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Theme: Development and regeneration

Topic: Nutritional and prenatal factors

Keywords: Chronic hypertension; Neuronal membrane; Polyunsaturated fatty acid; Spatial learning

## 1. Introduction

The cardiovascular protective effects of long chain n-3 polyunsaturated fatty acids (PUFAs) derived from fish oil have been repeatedly demonstrated by both human epidemiological and experimental animal studies. The consumption of an n-3 PUFA rich diet was shown to successfully reduce blood pressure in developing hypertension,

decrease coronary heart disease, and suppress cardiac arrhythmia [3,12,16,21,22,26,29,31,34,35]. In particular, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) were identified as the biologically active n-3 PUFA constituents in fish oil, of which DHA emerged as the potentially more effective substance [26,29].

Besides moderating hypertension, dietary n-3 PUFA intake in spontaneously hypertensive rats (SHRs) was shown to change tissue phospholipid composition in several organs including the brain [11,12,43,45]. After dietary treatment with perilla oil or linseed oil rich in the essential alpha-linolenic acid (ALA, 18:3 n-3), the (n-6)/

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(n-3) PUFA ratio in SHR brain membranes followed the ratio in the diets, and was reduced accordingly [11,43].

Interestingly, the balance between n-3 and n-6 PUFAs in cerebral membranes appeared to influence brain function, especially learning and memory processes. Normotensive rodents chronically supplemented with n-3 PUFA-enriched diets demonstrated enhanced spatial learning, which was accompanied by elevated brain DHA content [37,48]. The authors proposed that the changes in brain fatty acid composition could modify membrane fluidity by decreasing cholesterol level, which probably accounted for enhanced cognition [47,48]. At the same time, other studies also demonstrated that n-3 PUFA feeding increased neurotransmitter receptor density for dopamine and acetylcholine [4,14], both known for their prominent role in learning and memory processes.

In the present study, we aimed at identifying the behavioral correlates of alleviated hypertension after chronic n-3 PUFA-enriched dietary treatment of SHRs. Since spatial learning performance of SHR animals appeared to be impaired [17,30], we postulated that dietary n-3 PUFA supplementation could improve memory capacity not only by relieving hypertension, but also by being incorporated into cerebral membranes. In order to tackle the parallel vascular and behavioral effects of n-3 PUFA feeding in SHRs, we measured systolic blood pressure, assessed behavioral profile and analyzed fatty acid composition of cerebral membranes. Further, the different read-outs were correlated to unravel a potential, individual relationship between brain membrane composition, learning capacity and blood pressure in alleviated hypertension.

## 2. Materials and methods

### 2.1. Dietary design

The experimental diets were similar to those used in previous studies [8,14]. The control food was essentially identical to the standard rat chow produced by Hope Farms (Woerden, The Netherlands). All three diets consisted of the same amount of carbohydrates, proteins and minerals and contained the same calorie value. Diet 1 and Diet 2 were enriched by PUFAs, antioxidants, vitamins and particular extra additives. Table 1 shows the source of fatty acid additives and PUFA contents of the diets, while Table 2 summarizes the types and amounts of the other supplements.

### 2.2. Experimental groups

Male SHRs and normotensive Wistar–Kyoto (WKY) rats of 4 weeks of age were purchased from Harlan Nederland. The rats were randomly assigned to the three dietary groups. The combination of rat strains and diets produced six experimental groups of 10 animals each. The

Table 1

The source of fatty acid additives and polyunsaturated fatty acid content of the experimental diets (g/100 g)

Component	Control	Diet 1	Diet 2
Soybean oil (Florin, Switzerland)	5	2.5	2.5
Marinol C45 (source of EPA and DHA) (Loders Crocklaan, The Netherlands)	–	2.15	2.15
Ropufa 50 (source of AA) (Roche, The Netherlands)	–	0.35	0.35
Fatty acids			
18:2 n-6 (LA)	0.640	1.321	1.661
20:4 n-6 (AA)	0.000	0.118	0.118
Total n-6	0.640	1.439	1.779
18:3 n-3 (ALA)	0.155	0.137	0.184
20:5 n-3 (EPA)	0.000	0.589	0.589
22:6 n-3 (DHA)	0.000	0.382	0.382
Total n-3	0.155	1.108	1.155
<b>(n-6)/(n-3)</b>	<b>4.13</b>	<b>1.30</b>	<b>1.54</b>

animals were first group-housed in cages of five, and moved to individual cages at the age of 24 weeks, when spatial learning testing started. The rats were following the given nutritional regimes from 4 to 80 weeks of age, and were offered food and water ad libitum except the periods of the holeboard learning test, when food restriction was imposed. The animals were weighed weekly during the entire experiment and daily during the period of food restriction (Fig. 1).

### 2.3. Blood pressure measurement

Systolic blood pressure (BP) was repeatedly measured by the tail-cuff method [5,27] at the ages of 53, 54, 55, and 79 weeks. The rats were lightly anesthetized by isoflurane gas. Blood pressure was measured in each case three consecutive times and the values were averaged.

Table 2

Additional supplements of the experimental diets (g/100 g)

Nutrient	Component	Control	Diet 1	Diet 2
Antioxidants and vitamins	β-Carotene		0.02	0.02
	Flavonoids		0.2	0.2
	Folate	0.0004	0.001	0.001
	Selenium	0.000019	0.00004	0.00004
	Vitamin B6	0.00153	0.00172	0.00172
	Vitamin B12	0.00005	0.00012	0.00012
	Vitamin C		0.2	0.2
	Vitamin E	0.0063	0.3	0.3
Other	L-Acetylcarnitin			0.6
	Choline	0.15	0.15	0.4
	Phosphatidylcholine			0.2
	Phosphatidylserine			0.2
	Co-Q10			0.2
	Thiamin	0.002	0.002	0.2
	Tyrosine	0.944	0.944	1
	Tryptophan	0.232	0.232	1

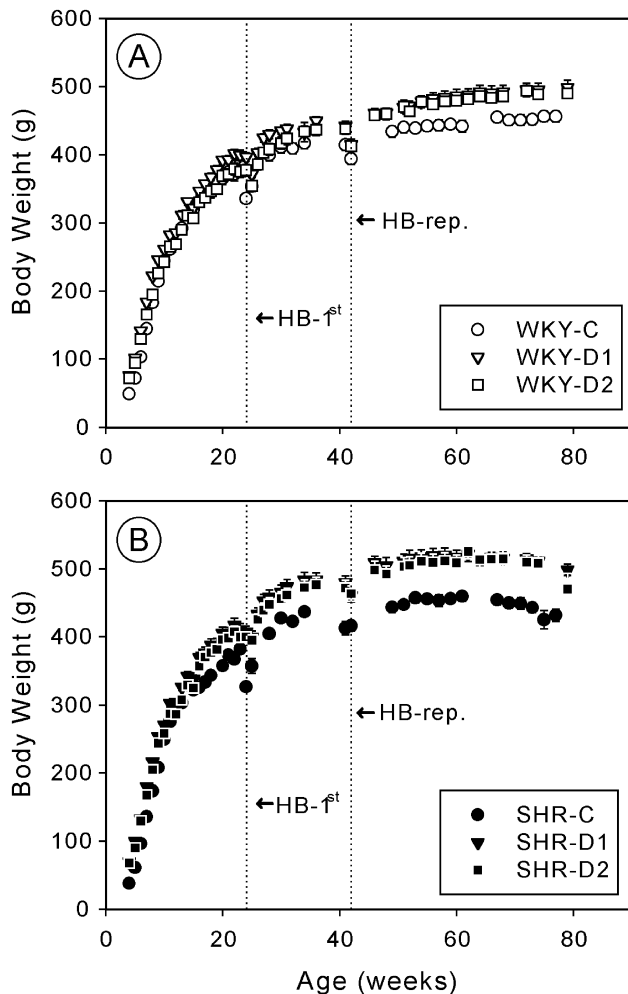


Fig. 1. Growth curves of the experimental groups [ $F(\text{diet})=15.209^{**}$ ,  $F(\text{bp})=7.947^{*}$ ]. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, HB: holeboard, SHR: spontaneously hypertensive rat strain, WKY: Wistar–Kyoto rat strain.

#### 2.4. Behavioral and learning tests

A small open field paradigm was used for the observation of spontaneous activity in a new environment. At the age of 19 weeks, the rats were moved from their home cage to a small open field (25×25×30 cm) in a dimly lit room, and their behavior was followed for 60 min. The actual behavioral pattern of the individual rats was registered every 10 s. The following behavioral patterns were recorded: sniffing, ambulation, digging, rearing, grooming and immobility. Scores were calculated to represent the frequency of the exhibited behavioral patterns. The calculation was carried out for consecutive 10-min time blocks and for the total 60-min observation period (1 point per manifested pattern per every 10 s).

Next, the animals were trained in the holeboard spatial discrimination task. The detailed experimental procedure has been described previously [9,41]. Briefly, the floor of the testing box contained 16 small removable pits in rows

of four, where chocolate chips could be placed. The rats were deprived of food 24 h prior to the start of the training, and had free access to their assigned diets only for 2 h per day after completing the task. The training started at the age of 24 weeks with habituation trials on 3 consecutive days (two trials per day). Subsequently, two training trials (4 h apart) were performed on 7 consecutive days. During the training trials, only four pits were baited arranged in a fixed pattern. The rats had to learn to visit only the baited holes once during the individual trials. The animals had 3 min to complete the task, or were removed from the holeboard when all four baited holes were visited. Reference memory score was calculated for each trial as: [(No. of visits to baited holes)+(No. of revisits to baited holes×0.5)] divided by [(4–No. of visits to baited holes)+(No. of visits and revisits to non-baited holes+No. of revisits to baited holes)]. The scores of the two daily trials were averaged and corrected for the value of the first training day. The test was repeated at the age of 41 weeks.

At the age of 44 weeks, the animals were trained in the Morris water maze spatial memory test [8]. The maze consisted of a water tank and a square, hidden platform on a fixed location, 1 cm beneath water level. A video camera recording the animals' swimming pattern was directed to a computer equipped with a videotracking software (EthoVision 2.0; Noldus, Wageningen, The Netherlands), which measured swimming distance and time, and also discriminated a platform area ( $d=48.5$  cm). The animals were trained twice a day on 5 consecutive days during the acquisition phase. The rats entered the water at four

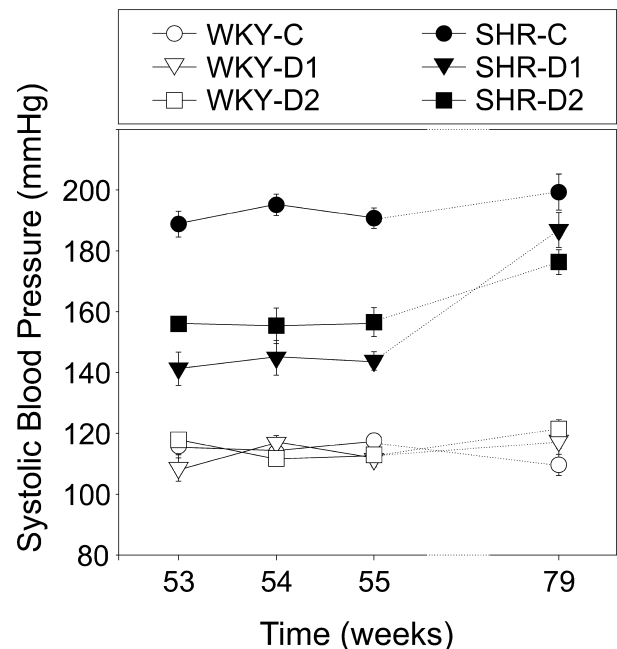


Fig. 2. The systolic blood pressure of the experimental groups [ $F(\text{diet})=41.538^{**}$ ,  $F(\text{bp})=880.784^{**}$ , interaction:  $F=39.917^{**}$ ]. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, SHR: spontaneously hypertensive rat strain, WKY: Wistar–Kyoto rat strain.

random starting points, were given 2 min to locate the platform, and were allowed to sit on the platform for 15 s. The average swimming distance of the two trials was calculated as daily score. A probe trial took place 3 days after the last acquisition trial, where the platform was removed from the pool. The rats were allowed to swim for 1 min and the time spent in the platform area was recorded as a measure for retention of the platform location.

### 2.5. Cerebral fatty acid analysis

At the age of 80 weeks, the rats were shortly perfused by an ice-cold saline solution of 5.8 mM EDTA following deep anesthesia with sodium pentobarbital. Lipids were extracted from whole brain according to Folch et al. [15]. The lipid classes were separated on thin layer chromatography plates (20×20, Silica G 60) using methyl acetate:isopropanol:chloroform:methanol:0.25% KCl in a ratio of 25:25:25:10:9. Lipids were transmethylated in presence of absolute methanol containing 5% HCl. Fatty acid methyl esters were determined by gas chromatography using a NUKOL capillary column (30 m×0.32 mm, 0.25

μm film thickness, Supelco, catalog No. 24131). To identify peaks, Supelco fatty acid standards were used (catalog Nos. 4-7085-U, 4-7015-U). The amount of the identified fatty acids was calculated as percentage of the total amount for each phospholipid class.

### 2.6. Statistical analysis

The growth curves, systolic blood pressure values and the behavioral results were compared by repeated measurement two-way analysis of variance (ANOVA). The Morris maze probe trial was tested by univariate two-way ANOVA. The brain fatty acid composition was analyzed by a multivariate general linear model two-way ANOVA. In all cases, the LSD correction was chosen for the post hoc multiple comparison analysis of dietary effect. We used the  $F$  values to express the dietary and blood pressure effects [ $F(\text{diet})$  and  $F(\text{bp})$ , respectively], where superscripted asterisks indicated significance ( $P$  values: \*  $\leq 0.05$ , \*\*  $\leq 0.01$ ). Correlation analysis was performed by the Pearson one-tailed test.

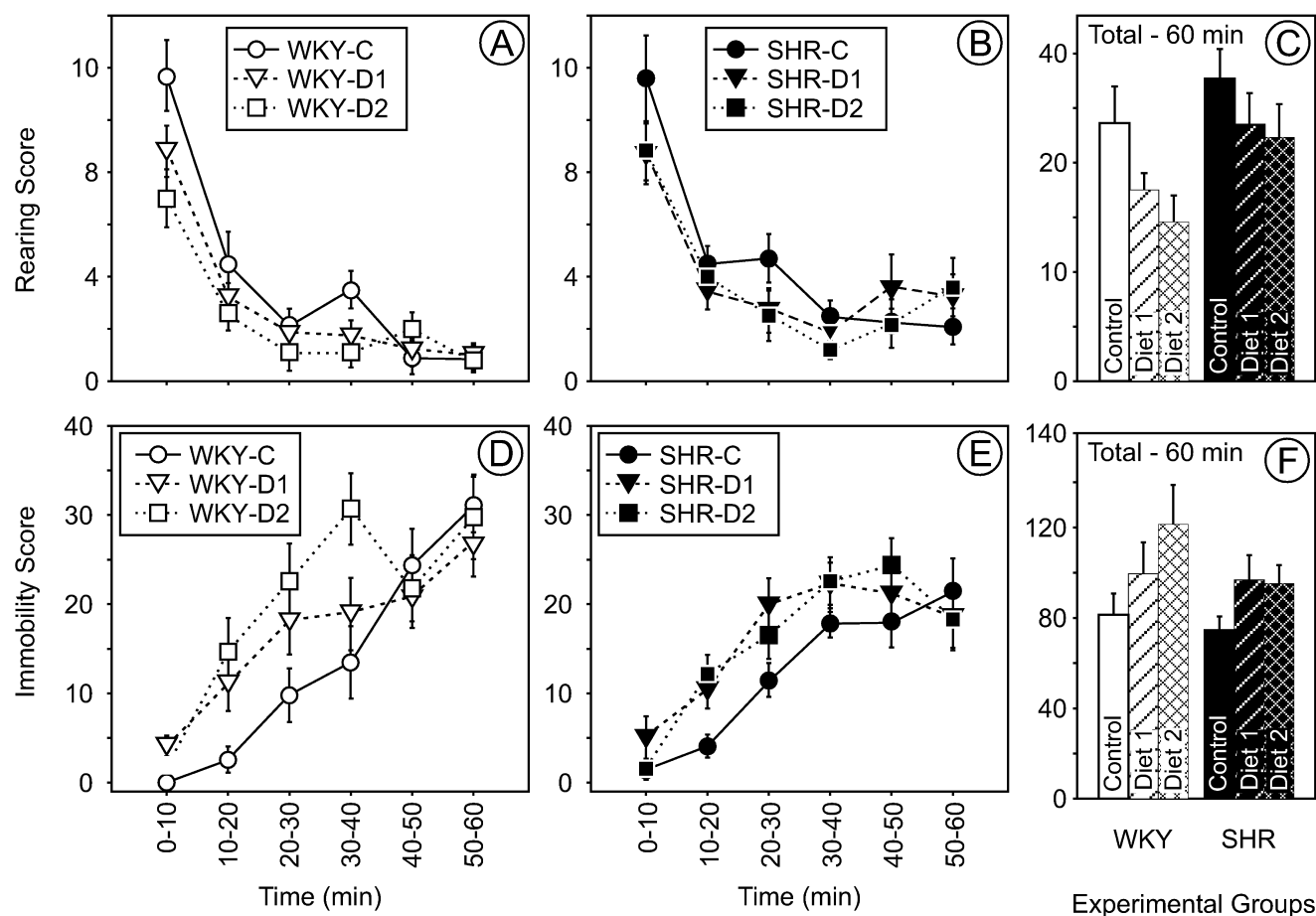


Fig. 3. Graphs of the small open field test. Panels A, B and C show the rearing scores [ $F(\text{diet})=3.743^*$ ,  $F(\text{bp})=6.916^*$ ], while panels D, E and F demonstrate the immobility scores [ $F(\text{diet})=3.763^*$ ,  $F(\text{bp})=1.649$ ]. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, SHR: spontaneously hypertensive rat strain, WKY: Wistar–Kyoto rat strain.



### 3. Results

The systolic BP of adult SHR (53–55 weeks) on control diet was about 50 mmHg higher than that of WKY rats. Dietary supplementation did not change the BP of WKY rats, while both Diet 1 and Diet 2 significantly reduced BP with 30–50 mmHg in adult SHR. The dietary effect on BP in SHR diminished with age (79 weeks) (Fig. 2).

Both hypertension and the experimental diets affected spontaneous activity in the small open field. The young adult SHR spent significantly longer time with rearing [ $F(\text{bp})=6.916^*$ ], grooming [ $F(\text{bp})=15.018^{**}$ ] and digging [ $F(\text{bp})=22.297^{**}$ ] than the WKY rats. Particularly Diet 2 reduced the activity of the animals, which was reflected by less frequent ambulation [ $F(\text{diet})=11.903^{**}$ ], rearing [ $F(\text{diet})=3.743^*$ ] and grooming [ $F(\text{diet})=3.714^*$ ], as well as longer periods of immobility [ $F(\text{diet})=3.763^*$ ] (Fig. 3).

Fig. 4 demonstrates spatial learning in the holeboard. The learning curves show diet-induced improvement in the performance of WKY rats, which was statistically signifi-

cant in the first session. Post hoc analysis revealed a specific effect of Diet 2 alone. In the water maze, adult SHR performed significantly worse than WKY rats both at the acquisition trials [ $F(\text{bp})=71.737^{**}$ ] and the retention trial [ $F(\text{bp})=17.988^{**}$ ]. Dietary supplementation did not improve spatial learning in the Morris maze.

Tables 3–5 summarize the hypertension and diet-induced changes in cerebral fatty acid composition of membrane phospholipids. In summary, saturated fatty acid content was not affected by blood pressure, but was remarkably reduced by the experimental diets in the phosphatidylserine and phosphatidylcholine fractions (16:0 and 18:0, respectively). The percentage of several n-7 and n-9 MUFA types was lower in SHR than in WKY rats, and was consistently elevated by the diets. Both n-3 and n-6 PUFA content significantly decreased in SHR compared to WKY rats most markedly in the phosphatidylinositol class, while the dietary supplementation considerably elevated both n-3 and n-6 PUFA concentration. An increase in total PUFA content was the most consistent across phospholipid classes. The (n-6)/(n-3) PUFA ratio was significantly reduced by the diets, most strikingly in the phosphatidylethanolamine class.

Correlation analysis demonstrated that the (n-6)/(n-3) PUFA ratio rather than DHA or AA contents alone corresponded with BP, which was especially obvious in SHR (Fig. 5A). The ratio of (n-6)/(n-3) PUFA in the phosphatidylethanolamine class stood in direct correlation with learning score in the holeboard test (Fig. 5B).

### 4. Discussion

Our BP measurements verified previous reports that increased dietary n-3 PUFA content effectively attenuated the development of hypertension in SHR [3,12,16]. The data indicated that the effect was apparent only in adult SHR and not in WKY rats, and disappeared at the end of the animals' life span. To date, BP changes with respect to dietary PUFA supplementation were not reported at this advanced age. We suggest that an age-related, general failure in BP regulation could have overruled the dietary effect at 79 weeks.

The SHR showed more active exploratory behavior in a novel environment, which had been repeatedly documented and attributed to increased locomotor activity in other open field paradigms [7,18,23,39,40]. The lack of correlation between exploratory behavior and BP also confirms previous data: although telemetric recording detected greater pressor response and tachycardia of SHR than of WKY rats during exploration, the magnitude of the autonomic responses appeared to be unrelated to behavior [40].

In the present study, Diet 1 led only to a subtle repression of activity in the small open field, while Diet 2

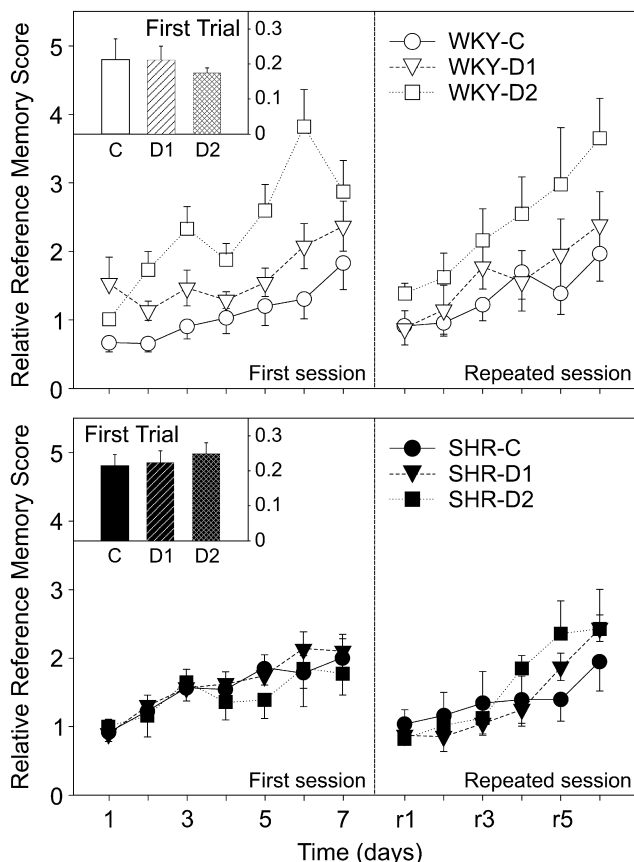


Fig. 4. Memory score in the holeboard spatial discrimination test [first session:  $F(\text{diet})=3.727^*$ ,  $F(\text{bp})=3.094$ ; repeated session:  $F(\text{diet})=2.078$ ,  $F(\text{bp})=1.668$ ]. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, SHR: spontaneously hypertensive rat strain, WKY: Wistar-Kyoto rat strain.

Table 3  
Fatty acid concentration of brain structural phospholipids in WKY rats

FA type	Fatty acids	Phospholipid family											
		PE			PS			PC			PI		
		WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2	WKY-C	WKY-D1	WKY-D2
DMA	16:0	5.16±0.76	5.72±0.74	4.81±1.20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	6.56±1.17	7.31±1.22	6.30±1.30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	5.38±1.00	6.47±1.15	5.30±1.70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	2.92±0.44	2.86±0.46	2.45±0.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total DMA	20.02±3.29	22.37±3.54	18.86±4.94	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	14:0	1.43±0.23	1.00±0.38	1.14±0.47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:0	n.d.	n.d.	n.d.	11.45±4.25	4.90±1.34	5.03±2.12	35.88±2.56	37.42±1.61	36.27±2.11	23.05±5.91	15.84±4.45	19.80±2.13
	18:0	14.31±3.07	11.98±1.53	13.47±1.59	33.40±4.25	36.06±1.48	35.89±1.53	17.67±2.22	13.99±1.18	15.33±2.15	28.15±6.72	26.23±5.05	32.00±3.24
	22:0	n.d.	n.d.	n.d.	0.99±0.25	1.19±0.07	1.09±0.14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total SFA	15.17±3.31	1.20±1.23	12.32±1.36	45.46±7.00	42.15±1.65	41.83±2.07	53.55±2.25	51.41±1.79	51.60±3.30	11.49±4.54	42.06±9.04	51.80±4.01
MUFA	14:1 n-5	1.20±0.23	0.59±0.27	0.76±0.28	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:1 n-7	0.95±0.74	1.49±0.54	2.11±0.70	n.d.	n.d.	n.d.	1.93±0.31	1.46±0.26	1.10±0.50	3.34±0.20	2.79±0.94	1.79±0.16
	18:1 n-7	3.85±0.40	4.38±0.29	4.26±0.22	n.d.	n.d.	n.d.	6.89±0.94	7.89±0.70	7.75±0.62	2.71±0.41	3.24±0.44	2.84±0.70
	18:1 n-9	17.26±1.19	17.52±0.80	17.05±0.48	20.37±2.86	27.29±2.66	27.12±3.85	22.94±1.76	24.36±1.25	24.04±1.50	13.39±3.30	9.08±2.38	9.41±2.52
	20:1 n-7	0.64±0.59	1.13±0.09	1.15±0.12	n.d.	n.d.	n.d.	0.36±0.51	1.00±0.08	0.89±0.37	n.d.	n.d.	n.d.
	20:1 n-9	4.96±0.73	4.58±0.35	4.86±0.39	3.10±0.88	3.89±0.38	4.06±0.52	1.82±0.30	1.60±0.08	1.75±0.16	n.d.	n.d.	n.d.
	22:1 n-9	n.d.	n.d.	n.d.	0.88±0.02	0.69±0.13	0.73±0.04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total MUFA	28.14±2.18	29.50±1.30	30.00±1.68	23.69±3.42	31.44±2.56	31.66±4.22	33.94±2.79	36.31±2.20	35.52±2.22	18.28±3.85	15.11±2.33	12.49±1.99
PUFA	16:3 n-4	1.22±0.47	0.93±0.13	1.07±0.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:2 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.06±0.71	1.04±0.27	1.08±0.57	5.49±1.55	2.63±1.60	2.77±1.06
	20:3 n-6	tr.	0.38±0.02	0.29±0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>20:4 n-6 (AA)</b>	<b>6.91±1.17</b>	<b>7.22±0.37</b>	<b>7.43±0.31</b>	<b>2.40±0.71</b>	<b>3.52±0.25</b>	<b>3.88±0.66</b>	<b>3.90±0.52</b>	<b>4.29±0.38</b>	<b>4.31±0.34</b>	<b>8.89±1.92</b>	<b>20.51±4.47</b>	<b>20.00±5.95</b>
	22:4 n-6	3.25±0.50	3.11±0.22	3.49±0.20	1.68±0.46	2.27±0.23	2.39±0.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22:5 n-3	tr.	0.56±0.06	0.40±0.05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>22:6 n-3 (DHA)</b>	<b>11.76±1.72</b>	<b>14.14±0.84</b>	<b>13.81±0.54</b>	<b>9.62±2.60</b>	<b>14.73±0.68</b>	<b>14.07±1.69</b>	<b>3.90±0.60</b>	<b>4.47±0.16</b>	<b>4.35±0.36</b>	<b>0.83±1.45</b>	<b>3.41±0.73</b>	<b>4.29±1.33</b>
	<b>Total PUFA</b>	<b>22.65±2.81</b>	<b>25.82±1.65</b>	<b>26.03±1.30</b>	<b>13.70±3.71</b>	<b>20.52±1.01</b>	<b>20.34±2.48</b>	<b>7.80±1.11</b>	<b>8.76±0.51</b>	<b>8.66±0.69</b>	<b>15.21±3.49</b>	<b>26.56±4.81</b>	<b>26.60±6.16</b>
	<b>(n-6)/(n-3)</b>	<b>0.86±0.05</b>	<b>0.73±0.03</b>	<b>0.78±0.02</b>	<b>0.43±0.04</b>	<b>0.39±0.02</b>	<b>0.45±0.03</b>	<b>1.30±0.25</b>	<b>1.19±0.05</b>	<b>1.24±0.13</b>	<b>11.49±4.54</b>	<b>7.02±1.83</b>	<b>5.36±1.12</b>
Rest		14.03±5.20	10.32±3.10	11.64±2.99	17.15±5.71	5.89±3.58	6.17±5.49	4.72±4.28	3.53±3.16	4.23±3.15	15.31±10.63	16.28±13.99	9.11±7.67

Data are given as mean±S.D. Bold face indicates fatty acid types that were manipulated in the experimental diets. Abbreviations: AA: arachidonic acid, C: control diet, D1: Diet 1, D2: Diet 2, DHA: docosahexaenoic acid, DMA: dimethylacetale (plasminogen-derived), FA: fatty acid, MUFA: monounsaturated fatty acid, n.d.: non-detected, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, WKY: Wistar–Kyoto rat strain.

Table 4  
Fatty acid concentration of brain structural phospholipids in SHR

FA type	Fatty acids	Phospholipid family											
		PE			PS			PC			PI		
		SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2	SHR-C	SHR-D1	SHR-D2
DMA	16:0	5.77±0.82	6.06±0.67	5.64±0.90	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	7.02±0.58	6.88±0.94	6.07±0.84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	4.65±0.86	5.35±0.84	4.96±1.19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	2.62±0.45	2.51±0.39	2.33±0.53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total DMA	20.07±2.43	20.80±2.73	19.00±3.23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	14:0	1.46±0.21	1.01±0.37	1.12±0.29	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:0	n.d.	n.d.	n.d.	11.75±5.58	7.48±0.51	6.94±2.20	36.80±2.27	35.87±2.98	33.66±2.81	18.67±3.95	19.03±3.76	17.98±4.35
	18:0	12.53±2.16	13.60±1.02	11.90±1.55	34.42±3.90	37.04±4.70	35.05±2.49	16.49±1.85	15.51±0.70	16.03±1.38	29.89±11.37	27.45±5.69	30.12±6.82
	22:0	n.d.	n.d.	n.d.	1.01±0.14	1.13±0.12	1.09±0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total SFA	13.81±2.52	14.69±1.28	13.03±1.60	47.18±7.93	45.08±4.71	42.95±3.54	53.29±2.92	51.39±2.85	49.68±1.98	48.56±15.30	46.48±9.13	48.10±10.54
MUFA	14:1 n-5	1.13±0.35	1.02±0.57	0.71±0.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:1 n-7	1.12±0.22	1.14±0.30	1.56±0.80	n.d.	n.d.	n.d.	1.39±0.62	1.78±0.58	1.53±0.32	2.91±1.31	3.90±1.33	2.41±1.38
	18:1 n-7	3.23±0.29	3.78±0.20	3.68±0.16	n.d.	n.d.	n.d.	5.98±0.55	6.07±0.56	6.01±0.24	1.95±0.79	2.73±0.47	2.58±0.52
	18:1 n-9	16.60±1.58	16.88±0.41	17.82±0.72	18.09±2.64	24.49±2.25	22.32±2.02	23.71±2.08	23.01±1.57	23.96±1.03	9.31±4.42	9.17±1.34	7.63±2.27
	20:1 n-7	0.64±0.28	0.91±0.10	0.93±0.10	n.d.	n.d.	n.d.	0.56±0.25	0.71±0.40	0.73±0.06	n.d.	n.d.	n.d.
	20:1 n-9	5.23±0.73	4.63±0.38	5.45±0.52	2.85±0.74	3.14±0.39	3.05±0.46	1.60±0.15	1.62±0.68	1.55±0.12	n.d.	n.d.	n.d.
	22:1 n-9	n.d.	n.d.	n.d.	0.59±0.04	tr.	0.63±0.08	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total MUFA	27.67±2.42	27.84±1.00	30.06±1.85	21.09±3.02	27.64±2.34	25.93±2.40	33.23±2.14	33.18±2.50	33.77±1.05	14.17±5.78	15.80±1.30	12.62±2.97
PUFA	16:3 n-4	1.61±0.37	1.05±0.22	1.15±0.38	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:2 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.08±0.50	1.41±0.32	1.37±0.23	3.45±1.92	2.62±1.27	2.50±1.08
	20:3 n-6	0.61±0.09	0.40±0.18	0.28±0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>20:4 n-6 (AA)</b>	<b>7.13±0.70</b>	<b>6.68±0.42</b>	<b>7.40±0.58</b>	<b>2.02±0.59</b>	<b>3.13±0.47</b>	<b>3.21±0.54</b>	<b>4.06±0.57</b>	<b>3.45±0.15</b>	<b>4.12±0.26</b>	<b>5.54±1.67</b>	<b>11.22±3.67</b>	<b>13.85±5.15</b>
	22:4 n-6	3.38±0.35	2.83±0.23	3.41±0.32	1.69±0.49	1.61±0.85	2.03±0.27	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22:5 n-3	tr.	0.40±0.03	0.32±0.07	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>22:6 n-3 (DHA)</b>	<b>11.61±1.40</b>	<b>12.70±0.77</b>	<b>13.00±0.97</b>	<b>8.35±2.81</b>	<b>13.99±2.54</b>	<b>12.26±1.92</b>	<b>3.38±0.49</b>	<b>3.21±0.28</b>	<b>3.99±0.26</b>	<b>0.39±0.37</b>	<b>1.19±0.75</b>	<b>2.41±0.85</b>
	<b>Total PUFA</b>	<b>23.68±2.01</b>	<b>23.45±1.01</b>	<b>25.15±2.14</b>	<b>12.06±3.86</b>	<b>18.73±2.68</b>	<b>17.49±2.61</b>	<b>7.44±1.03</b>	<b>6.66±0.35</b>	<b>8.10±0.49</b>	<b>9.37±2.26</b>	<b>15.04±3.64</b>	<b>18.75±5.65</b>
	<b>(n-6)/(n-3)</b>	<b>0.92±0.05</b>	<b>0.75±0.03</b>	<b>0.83±0.03</b>	<b>0.45±0.03</b>	<b>0.35±0.07</b>	<b>0.43±0.03</b>	<b>1.54±0.22</b>	<b>1.53±0.21</b>	<b>1.38±0.08</b>	<b>11.75±2.74</b>	<b>10.22±3.16</b>	<b>7.04±1.05</b>
Rest		14.78±4.26	13.23±3.11	12.76±3.24	19.68±7.13	8.56±8.44	13.63±4.83	6.03±2.88	8.78±5.24	8.45±2.62	27.90±7.82	22.69±10.54	20.52±10.07

Data are given as mean±S.D. Bold face indicates fatty acid types that were manipulated in the experimental diets. Abbreviations: AA: arachidonic acid, C: control diet, D1: Diet 1, D2: Diet 2, DHA: docosahexaenoic acid, DMA: dimethylacetale (plasminogen-derived), FA: fatty acid, MUFA: monounsaturated fatty acid, n.d.: non-detected, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, SHR: spontaneously hypertensive rat strain, tr.: trace.



Table 5  
Statistical *F* values of fatty acid concentration analysis in brain structural phospholipids

FA type	Fatty acids	Phospholipid family											
		PE			PS			PC			PI		
		Diet effect	BP effect	Interaction	Diet effect	BP effect	Interaction	Diet effect	BP effect	Interaction	Diet effect	BP effect	Interaction
Overall effect		34.678**	13.573	0.492	2.140	1.922	5.008*	4.463**	23.438**	3.149**	5.450**	8.741**	2.048*
DMA	16:0	1.723	4.876*	2.567	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:0	1.814	0.260	2.414	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-7	2.173	0.584	2.309	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:1 n-9	1.565	0.045	2.422	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total DMA	2.533	0.229	0.334	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SFA	14:0	0.744	0.151	1.767	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:0	n.d.	n.d.	n.d.	6.133*	0.949	0.055	2.203	2.346	2.182	0.863	0.015	1.732
	18:0	0.208	1.863	4.867*	6.706**	0.838	3.455	7.505**	0.510	2.611	0.906	0.380	0.526
	22:0	n.d.	n.d.	n.d.	0.582	0.311	1.187	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total SFA	1.811	0.254	4.239*	2.262	1.480	0.109	4.907*	0.958	0.653	1.518	0.040	0.699
MUFA	14:1 n-5	3.523	0.184	1.322	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	16:1 n-7	1.927	4.027	0.328	n.d.	n.d.	n.d.	2.911	0.303	5.450**	2.496	0.683	1.415
	18:1 n-7	8.491**	40.184**	0.551	n.d.	n.d.	n.d.	3.080	62.868**	2.367	5.667**	2.663	2.112
	18:1 n-9	7.946**	0.138	2.270	5.643*	10.158**	0.298	0.737	0.240	1.840	4.313*	2.364	2.371
	20:1 n-7	9.335**	23.298**	1.147	n.d.	n.d.	n.d.	6.899**	0.848	2.368	n.d.	n.d.	n.d.
	20:1 n-9	4.707*	0.541	0.572	0.372	2.769	1.444	0.406	2.238	0.770	n.d.	n.d.	n.d.
	22:1 n-9	n.d.	n.d.	n.d.	2.320	17.711**	4.736*	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total MUFA	6.106**	1.873	1.008	25.522**	19.288**	0.987	1.286	8.272**	1.166	5.185**	1.295	2.312
PUFA	16:3 n-4	6.721**	0.019	0.010	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	18:2 n-6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.553	2.746	0.580	5.025*	3.699	1.633
	20:3 n-6	27.375**	0.000	0.289	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>20:4 n-6 (AA)</b>	<b>4.000*</b>	<b>4.359</b>	<b>3.687</b>	<b>3.874*</b>	<b>3.087</b>	<b>0.102</b>	<b>3.132</b>	<b>6.164*</b>	<b>6.381**</b>	<b>17.458**</b>	<b>10.467**</b>	<b>3.068</b>
	22:4 n-6	8.574**	4.519	1.424	2.643	1.550	0.236	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	22:5 n-3	29.285**	14.520**	1.527	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	<b>22:6 n-3 (DHA)</b>	<b>1.937</b>	<b>10.505**</b>	<b>2.932</b>	<b>9.007**</b>	<b>2.086</b>	<b>0.032</b>	<b>7.552**</b>	<b>40.361**</b>	<b>6.113**</b>	<b>12.194**</b>	<b>20.608**</b>	<b>1.418</b>
	<b>Total PUFA</b>	<b>6.764**</b>	<b>1.848</b>	<b>3.303*</b>	<b>24.549**</b>	<b>5.584*</b>	<b>0.179</b>	<b>4.904*</b>	<b>21.061**</b>	<b>6.383**</b>	<b>18.233**</b>	<b>34.797**</b>	<b>1.432</b>
	<b>(n-6)/(n-3)</b>	<b>70.381**</b>	<b>16.337**</b>	<b>1.148</b>	<b>14.019**</b>	<b>1.258</b>	<b>3.028</b>	<b>1.834</b>	<b>23.992**</b>	<b>1.331</b>	<b>12.672**</b>	<b>4.140*</b>	<b>1.035</b>
Rest		2.414	2.267	0.398	14.562**	5.442*	0.783	0.302	11.428**	1.209	1.390	9.391**	0.354

Bold face indicates fatty acid types that were manipulated in the experimental diets. Abbreviations: AA: arachidonic acid, BP: blood pressure, C: control diet, D1: Diet 1, D2: Diet 2, DHA: docosahexaenoic acid, DMA: dimethylacetale (plasminogen-derived), FA: fatty acid, MUFA: monounsaturated fatty acids, n.d.: non-detected, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PI: phosphatidylinositol, PS: phosphatidylserine, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid.

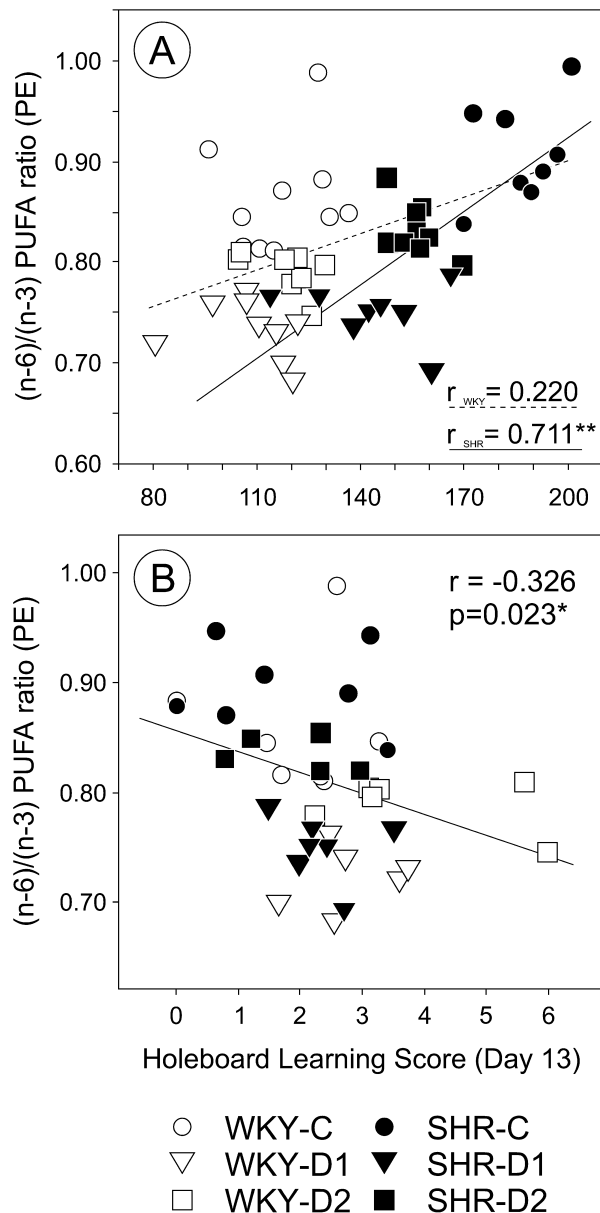


Fig. 5. (A) Correlation between systolic blood pressure on week 53 and (n-6)/(n-3) PUFA ratio in the phosphatidylethanolamine class of cerebral membranes. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, PE: phosphatidylethanolamine, SHR: spontaneously hypertensive rat strain, WKY: Wistar-Kyoto rat strain. (B) Correlation between holeboard learning score on day 13 and (n-6)/(n-3) PUFA ratio in the phosphatidylethanolamine class of cerebral membranes. Abbreviations: C: control diet, D1: Diet 1, D2: Diet 2, PE: phosphatidylethanolamine, SHR: spontaneously hypertensive rat strain, WKY: Wistar-Kyoto rat strain.

significantly reduced exploratory behavior. A similar tendency was observed in the holeboard in that specifically Diet 2 improved learning of WKY rats. Since the composition of Diet 2 included other additives than PUFAs, the additional supplements, rather than PUFAs alone must

have been responsible for this effect. The findings correspond with the conclusion of a previous study of ours, that increasing the dietary n-3 PUFA concentration above daily requirement, rather than as a compensation for dietary n-3 PUFA deficiency produces no conspicuous effect on the behavior of rats, when dietary groups are compared [8]. Therefore we propose that n-3 PUFA supplementation to hypertensives may not directly improve cognitive performance. Instead, n-3 PUFA load can moderate neuronal damage imposed by hypertension-related, acute cerebrovascular events, possibly by combating oxidative stress [46].

On the other hand, the correlation between spatial learning performance and the (n-6)/(n-3) PUFA ratio appeared to demonstrate subtle but sound PUFA-related improvement in learning capacity. Such correlation analysis appears to be a more sensitive method than group comparisons to reveal the concealed, dietary PUFA-induced improvement in learning, probably because the individual variations in spatial memory and brain fatty acid composition coincide.

The brain fatty acid analysis demonstrated that MUFA and PUFA concentrations were lower in SHRs than in WKY rats. Since hypertension is a vascular type of pathology, altered fatty acid transport through the blood-brain barrier (BBB) may be one factor to account for the hypertension-related difference. The endothelial cells of the BBB selectively transport PUFAs and their precursors, while the perivascular astrocytes actively participate in the metabolic elongation of fatty acids [10,28]. Although the effect of increasing BP on endothelial fatty acid transport has not been established, hypertension was shown to alter other carrier-mediated mechanisms like tryptophan and glutamic acid trafficking, which correlated with brain content [38]. Furthermore, perivascular astrocytic metabolism was also found to be compromised in hypertensive rats [44]. Therefore either pressure-induced endothelial dysfunction at the BBB or exhausted astrocytic metabolism could lead to reduced MUFA and PUFA content in SHRs. Nevertheless, an alternative explanation may identify an elevated lipid peroxidation accompanying high BP as the cause [20,32] since unsaturated fatty acids are particularly vulnerable for oxidative stress given the presence of less stable double bonds in the molecules [33].

Dietary PUFA supplementation increased brain PUFA concentration irrespective of specific PUFA type, and reduced (n-6)/(n-3) PUFA ratio. These data strongly correspond with the dietary composition, and reinforce that dietary fatty acid content is reflected in the phospholipid structure of cerebral membranes [6,24]. Cerebral MUFA concentration increased while SFA ratio decreased due to the experimental diets, the latter possibly on account of replacement by PUFA/MUFA.

Since Diet 1 and Diet 2 were enriched with PUFA at the expense of MUFA content, the consistent increase of

cerebral MUFA level across several subtypes was unexpected, and can find explanation only in cellular fatty acid biochemistry. The elevated MUFA concentration is specially intriguing in the light of the view that olive oil rich in MUFA (18:1 n-9) appears to be protective against atherosclerosis and can lower low-density lipoprotein (LDL) cholesterol [2,25].

Although the correlation found between BP and (n-6)/(n-3) PUFA ratio in SHR brain was prominent, it is still tempting to speculate that no direct causality operates between the two parameters. Dietary n-3 PUFA supplementation exhibits multifaceted biochemical actions extending from structural changes in membrane composition to competitive inhibition of the synthesis of signaling molecules like vasoactive prostaglandins [1]. Reduced serum concentration of thromboxanes or modulation of the calcium homeostasis of vascular smooth muscle cells were suggested as mechanisms behind the BP lowering effect of DHA, rather than the improvement of neural blood pressure regulation [11,13,19,36,42]. Therefore we have come to the view that BP and cerebral (n-6)/(n-3) PUFA ratio coincide, but are functionally not directly related. However, an inverse reasoning cannot exclude that alleviation of hypertension (by n-3 PUFAs) is beneficial to cerebral membrane composition by allowing optimal trafficking and conversion of fatty acids at the BBB.

## 5. Conclusions

We conclude that chronically increased dietary PUFA intake at a reduced (n-6)/(n-3) ratio can very effectively prevent the development of hypertension. Therefore the regular consumption of fish oil-derived PUFAs is highly recommended to individuals who are prone to develop hypertension and related neurological disorders.

Furthermore, we have shown that cerebral fatty acid composition of membrane phospholipids follows dietary intake, which is not necessarily reflected in spontaneous learning and behavior. Instead, n-3 PUFA load in the brain may prove to be particularly beneficial when the system is severely challenged. Thus, the major cerebral implications of regular dietary n-3 PUFA consumption lies rather in fostering recovery and limiting neuronal damage in stroke and other acute cerebrovascular deficiency.

The vasoactive effects of increased PUFA intake can originate directly from PUFAs themselves, but we have demonstrated that a PUFA-rich diet drives MUFA concentration in membrane phospholipids also high. Since MUFAs are also biologically very active fatty acids, the beneficial working attributed to dietary n-3 PUFAs may, in fact, be achieved in concert with MUFAs. The contribution of metabolically generated MUFAs after PUFA supplementation to the control of hypertension renders further investigation.

## Acknowledgements

The study was financed by the Numico Dementia Project No. 98/006 and by Lipidiet EC No. QLRT-2001-00172.

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